

FIGURE 1: General Cloning Strategy

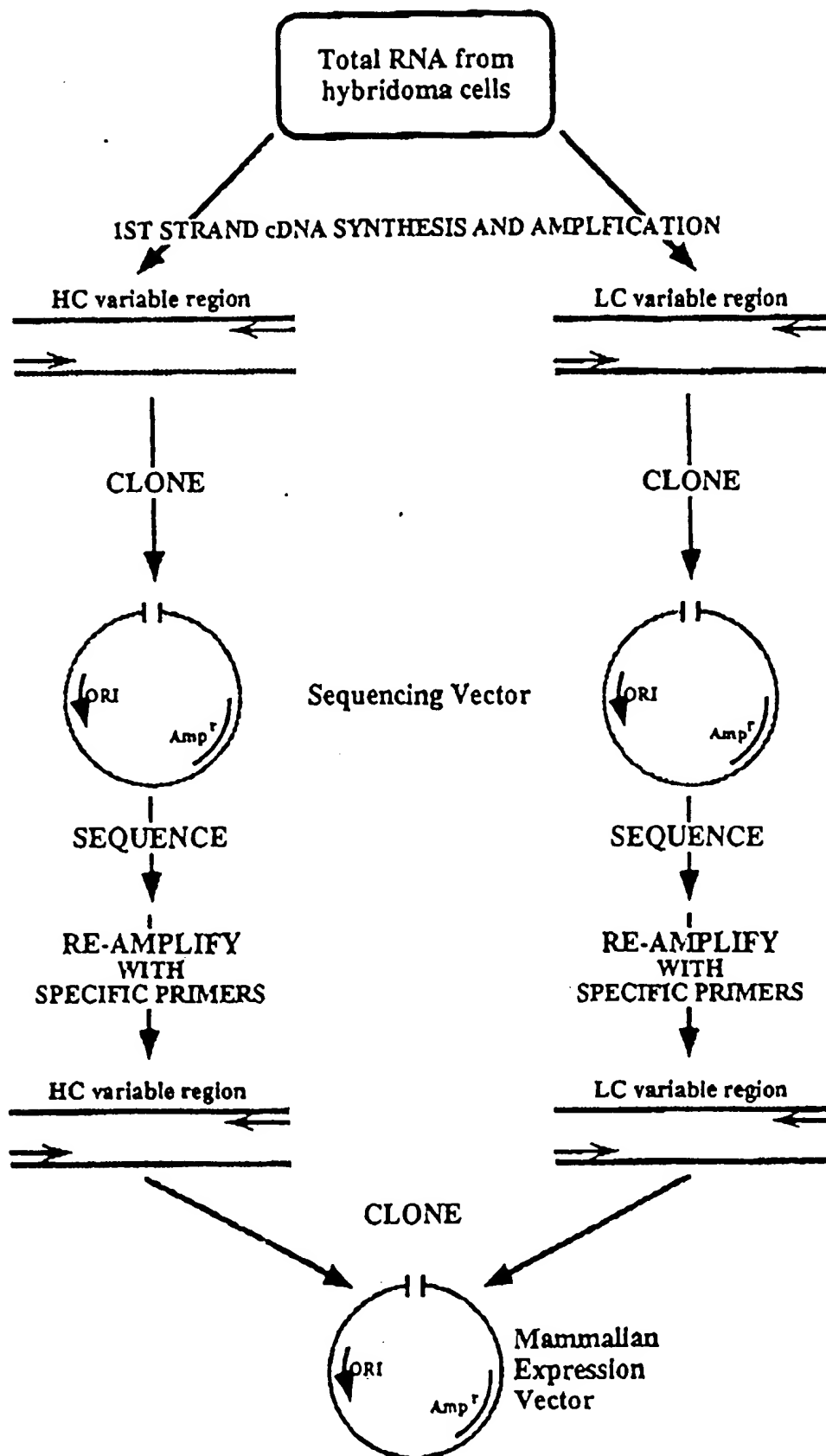


FIGURE 2

Mouse Heavy Chain "front" primers

JSS1

5'-ATTTTCAGGCCCAGCCGGCCATGGCCGARGTRMAGCTKSAKGAGWC-3'

SEQ ID NO. 1

JSS2

5'-ATTTTCAGGCCCAGCCGGCCATGGCCGARGTYCARCTKCARCARYC-3'

SEQ ID NO. 2

JSS3

5'-ATTTTCAGGCCCAGCCGGCCATGGCCCAGGTGAAGCTKSTSGARTC-3'

SEQ ID NO. 3

JSS4

5'-ATTTTCAGGCCCAGCCGGCCATGGCCGAVGTGMWGCTKGTGGAGWC-3'

SEQ ID NO. 4

JSS8

5'-ATTTTCAGGCCCAGCCGGCCATGGCCCAGGTBCARCTKMARSARTC-3'

SEQ ID NO. 5

Mouse Heavy chain "back" primers

JS300

5'-GAARTAVCCCTTGACCAGGC-3'

SEQ ID NO. 6

Mouse Light Chain Leader "front" primers

JSS009

5'-GGAGGCGGCGGTTCTGACATTGTGMTGWCMCARTC-3'

SEQ ID NO. 7

JSS010

5'-GGAGGCGGCGGTTCTGATRTTKYGATGACBCARRC-3'

SEQ ID NO. 8

JSS011

5'-GGAGGCGGCGGTTCTGAYATYMAGATGACMCAGWC-3'

SEQ ID NO. 9

JSS012

5'-GGAGGCGGCGGTTCTSAATTGWKCTSACYCAGTC-3'

SEQ ID NO. 10

Mouse Light Chain "back" primer

JS153

5'-TTCATAGGCGGCCGCACTAGTAGCMCGTTTCAGYTCCARC-3'

SEQ ID NO. 11

JS154

5'-TTCATAGGCGGCCGCACTAGTAGCMCGTTTKATYTCCARC-3'

SEQ ID NO. 12

OKA57

5'-GCACCTCCAGATGTAACTGCTC-3'

SEQ ID NO. 13

"13C4" Specific Primers

OKA144

5'-CTTGATCGCGACAGCTACAGGTGTCCACTCCCAGGTGCAGCTGCAGGAG-3'

SEQ ID NO. 14

OKA143

5'-GGTATGGAATTCTGAGGAGACTGTGAGAGTGGTGCC-3'

SEQ ID NO. 15

OKA145

5'-GGTCTGATATCGTGATGTCCCAGTCTCACAAATTC-3'

SEQ ID NO. 16

OKA146

5'-GACATATTCGAAAAGTGTACTTACGTTTCAGCTCCAGACTGG-3'

SEQ ID NO. 17

FIGURE 3

13C4 anti-STX1 heavy chain variable region

CAGGTGCAGCTGCAGGAGTCTGGGGCTGAGCTGGTGAGGTCTGGGGCCTCAGTGAGGATG SEQ ID NO. 18
Q V Q L Q E S G A E L V R S G A S V R M SEQ ID NO. 19

TCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAATATGCACTGGGTAAACAGACA
S C D A S G Y T F T S Y N M H W V K Q T

CCTGGACAGGGCCTGGAATGGATTGGATATATTTATCCTGGAAATGGTGGTACTAACTAC
P G Q G L E W I G Y I Y P G N G G T N Y

ATTGAGAAATTTAAGGGCAAGGCCATATTGACTGCAGACACATCCTCCAGCACAGCCTAC
I Q K F K G K A I L T A D T S S S T A Y

ATGCAGATCAGCAGTCTGACATCTGAAGACTCTGCGGTCTATTTCTGTACAAGAAGTCCCTCT
M Q I S S L T S E D S A V Y F C T R S P S

CACTACAGTAGTGACCCCTACTTTGACTACTGGGGCCAGGGCACCCTCTCACAGTCTCCTCA
H Y S S D P Y F D Y W G Q G T T L T V S S

13C4 anti-STX1 light chain variable region

GATATCGTGATGTCCAGTCTCACAAATTCATGTCCACATCAGTCGGAGACAGGGTCAGCATC SEQ ID NO. 20
D I V M S Q S H K F M S T S V G D R V S I SEQ ID NO. 21

ACCTGTAAGGCCAGCCAGGATGTGGGTACTGCTGTTGCCTGGTATCAGCAGAATCCAGGACAA
T C K A S Q D V G T A V A W Y Q Q N P G Q

TCTCCTAAATTTCTGATTTACTGGGCATCCACACGGCACACTGGAGTCCCTGATCGCTTCACA
S P K F L I Y W A S T R H T G V P D R F T

GGCAGTGGATCTGGGACAGATTTCACTCTCACCATACCAATGTGCAGTCTGAAGACTTGGCA
G S G S G T D F T L T I T N V Q S E D L A

GATTATTTCTGTCAGCAATATAGCAGTTATCCTCTCACGTTCCGGTGCTGGGACCAGTCTGGAG
D Y F C Q Q Y S S Y P L T F G A G T S L E

CTGAAACGT
L K R

FIGURE 4

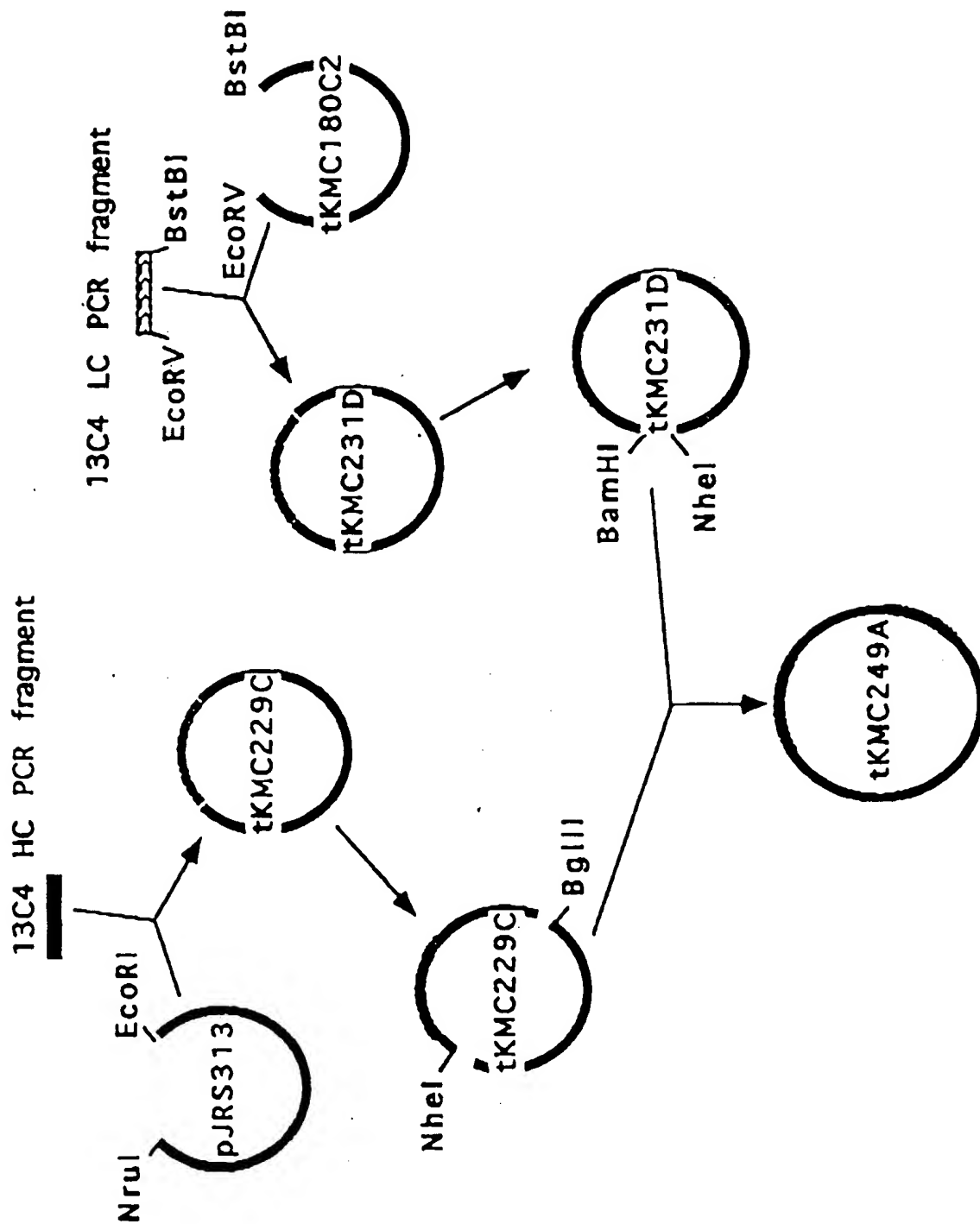


FIGURE 5

Mouse Heavy Chain "Front" Primers

JS001	5'-ATTT <u>CAGGCC</u> CAGCCGGCCATGGCCGARGTRMAGCTKSAKGAGWC-3'	SEQ ID NO. 22
JS002	5'-ATTT <u>CAGGCC</u> CAGCCGGCCATGGCCGARGTYCARCTKCARCARYC-3'	SEQ ID NO. 23
JS003	5'-ATTT <u>CAGGCC</u> CAGCCGGCCATGGCCAGGTGAAGCTKSTSGARTC-3'	SEQ ID NO. 24
JS004	5'-ATTT <u>CAGGCC</u> CAGCCGGCCATGGCCGAVGTGMWGCTKGTGGAGWC-3'	SEQ ID NO. 25
JS008	5'-ATTT <u>CAGGCC</u> CAGCCGGCCATGGCCAGGTBCARCTKMARSARTC-3'	SEQ ID NO. 26

Mouse Heavy Chain "Back" Primers

JS300	5'-GAARTAVCCCTTGACCAGGC-3'	SEQ ID NO. 27
JS160	5'-GCTGCCACCGCCACCTGMRGAGACDGTGASTGARG-3'	SEQ ID NO. 28
JS161	5'-GCTGCCACCGCCACCTGMRGAGACDGTGASMGTRG-3'	SEQ ID NO. 29
JS162	5'-GCTGCCACCGCCACCTGMRGAGACDGTGASCAGRG-3'	SEQ ID NO. 30

Mouse Light Chain Leader "Front" Primers

JS009	5'-GGAGGCGGCGGTTCTGACATTGTGMTGWCMCARTC-3'	SEQ ID NO. 31
JS010	5'-GGAGGCGGCGGTTCTGATRTTKYGATGACBCARRC-3'	SEQ ID NO. 32
JS011	5'-GGAGGCGGCGGTTCTGAYATYMAGATGACMCAGWC-3'	SEQ ID NO. 33
JS012	5'-GGAGGCGGCGGTTCTSAAATTGWKCTSACYCAGTC-3'	SEQ ID NO. 34

Mouse Light Chain "Back" Primers

JS153	5'-TTCATAGGCGGCCGCACTAGTAGCMCGTTTCAGYTCCARC-3'	SEQ ID NO. 35
JS154	5'-TTCATAGGCGGCCGCACTAGTAGCMCGTTTKATYTCCARC-3'	SEQ ID NO. 36

"11E10" Specific Primers

11E10HF	5'-ATATACT <u>CGCG</u> ACAGCTACAGGTGTCCACTCCGAAGTCCAAGTCAACAGCC-3'	SEQ ID NO. 37
11E10HB	5'-ATTAATGAATTCTGCGGAGACGGTGAGAGTGGTC-3'	SEQ ID NO. 38
11E10LF	5'-TTAAATGATATCGTGCTGTCACAATCTCC-3'	SEQ ID NO. 39
11E10LB	5'-TAATCGTT <u>CGAA</u> AGTGTACTTACGTTTCAGTTCAGCTTGGTCC-3'	SEQ ID NO. 40

	10	20	30	40	50	60	70	80
1 11E10 II	GAAGTCCAAC E V Q	TGCAACAGCC L Q Q	TGGACCTGAG P G P	CTGGAGAAGC L E K	CTGGCGCTTC P G A	AGTGAAACTA V K L	TCTTGCAAGG S C K	CTTCTGGTTA A S G Y
1 11E10 II	CTCTTTCACT S F T	GACTACAACA D Y N	TGAACTGGGT M N W	GAAGCAGAAC K Q N	AATGGAGAGA N G E	GCCTTGAGTG S L E	GATTTGGAAGA W I G	ATTGATCCCTT K I D P
1 11E10 II	ACTATGGTGG Y Y G	TCTTAGCTAC G P S	AACCAAGAAGT Y N Q	TCAAGGACAA F K D	GGCCACATTG K A T	ACTCTTAGACA L T V	AGTCTTCCAG D K S	CACAGCCTAC S S T A Y
1 11E10 II	ATGCAGTTCA M Q F	AGAGCC'NGAC K S L	ATCTGAGGAC T S E	TCTGCAGTCT D S A	ATTACTGTAC Y Y C	AAGAGGGGGA T R C	AATAGGGACT G N R	GGTACTTCGA D W Y F D
1 11E10 II	TGTGTGGGGC G CAGGGACCA	GGCTCACCGT CTCCGCA						

FIGURE 7

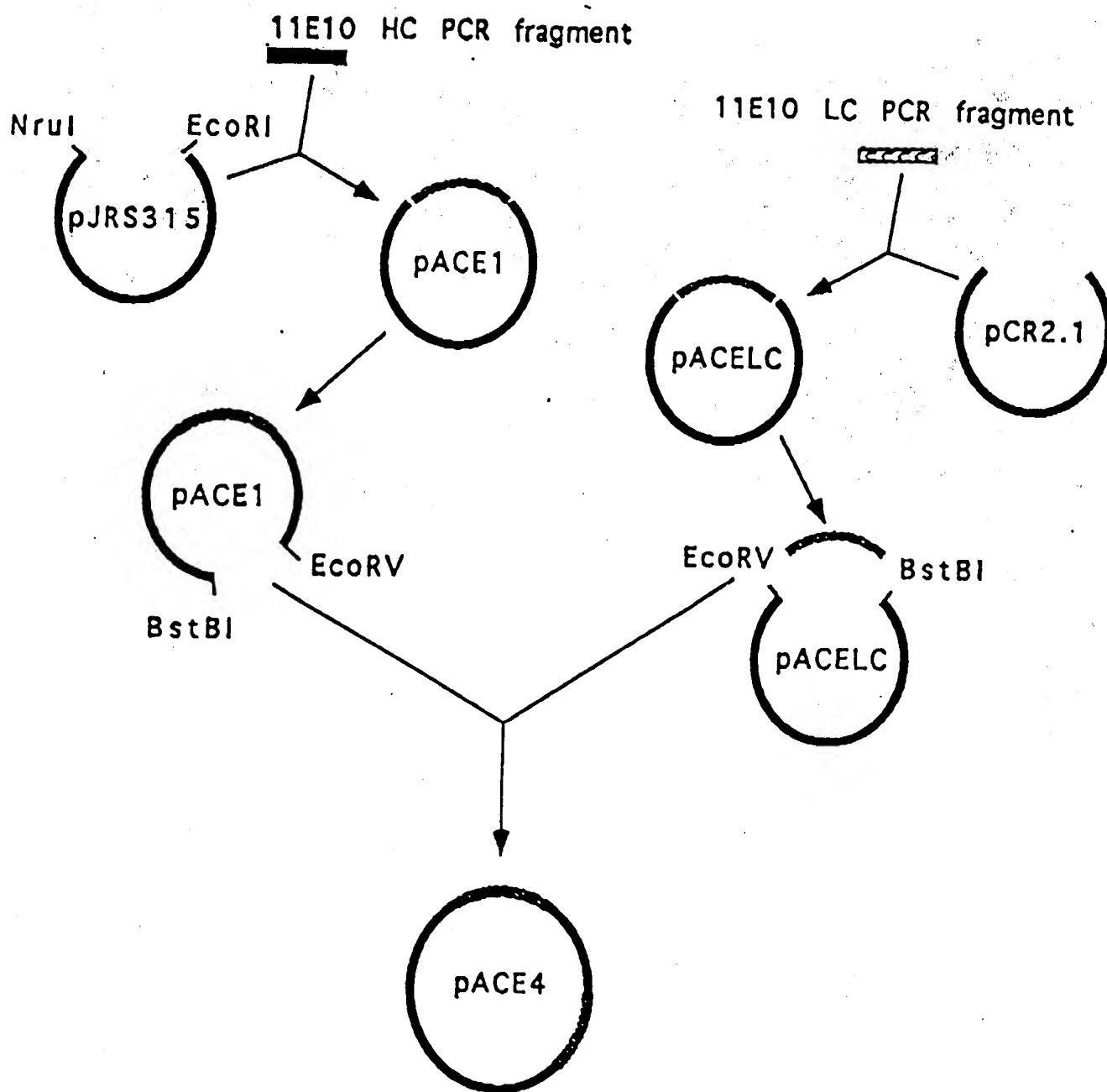
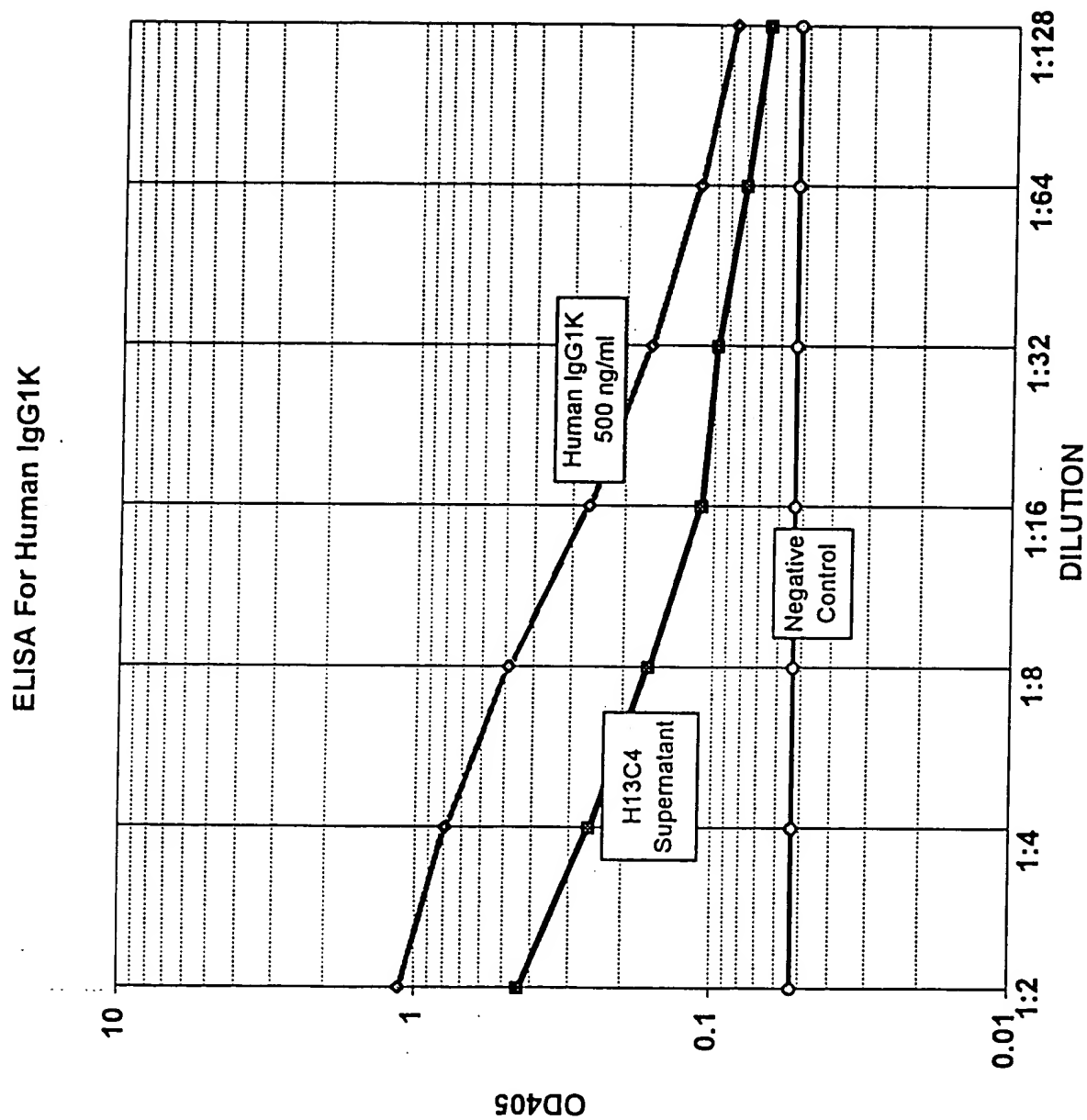
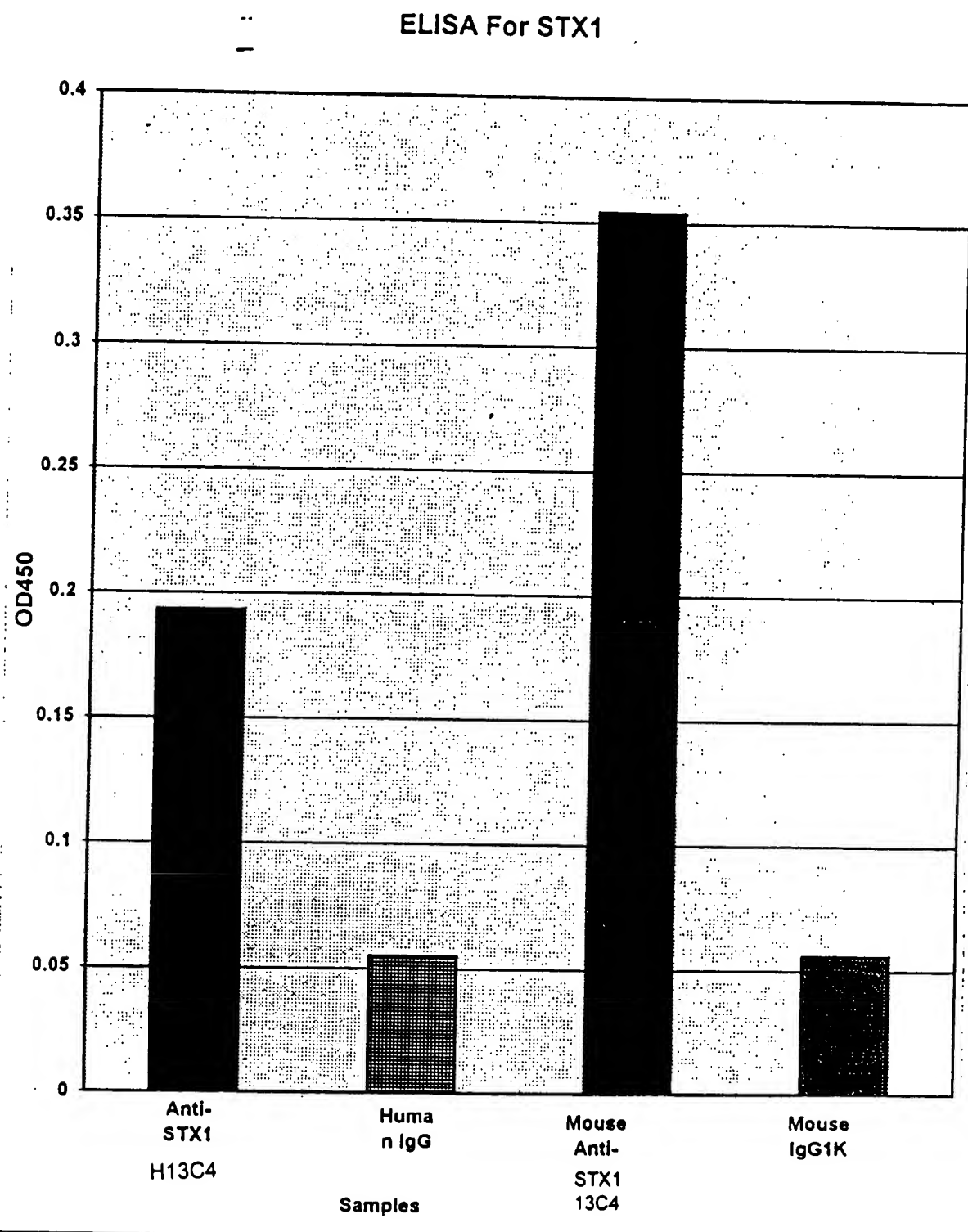


FIGURE 8



Chimeric Anti-STX1 H13C4, Human IgG1K, and culture medium (neg. control) were incubated on goat anti-human IgGfC coated microplates. The wells were probed with goat anti-human Kappa HRP conj. and reacted with ABTS. OD signals were read at 405 nm.

FIGURE 9



Chimeric Anti-STX1, Mouse Anti-STX, and Human and Mouse IgG1K were incubated on STX1 coated microplates. The wells were probed with either goat anti-human or anti-mouse IgG HRP conjugates. After reaction with TMB signals were read at 450 nm.

FIGURE 10 Anti-Stx2 Antibody Production ELISA

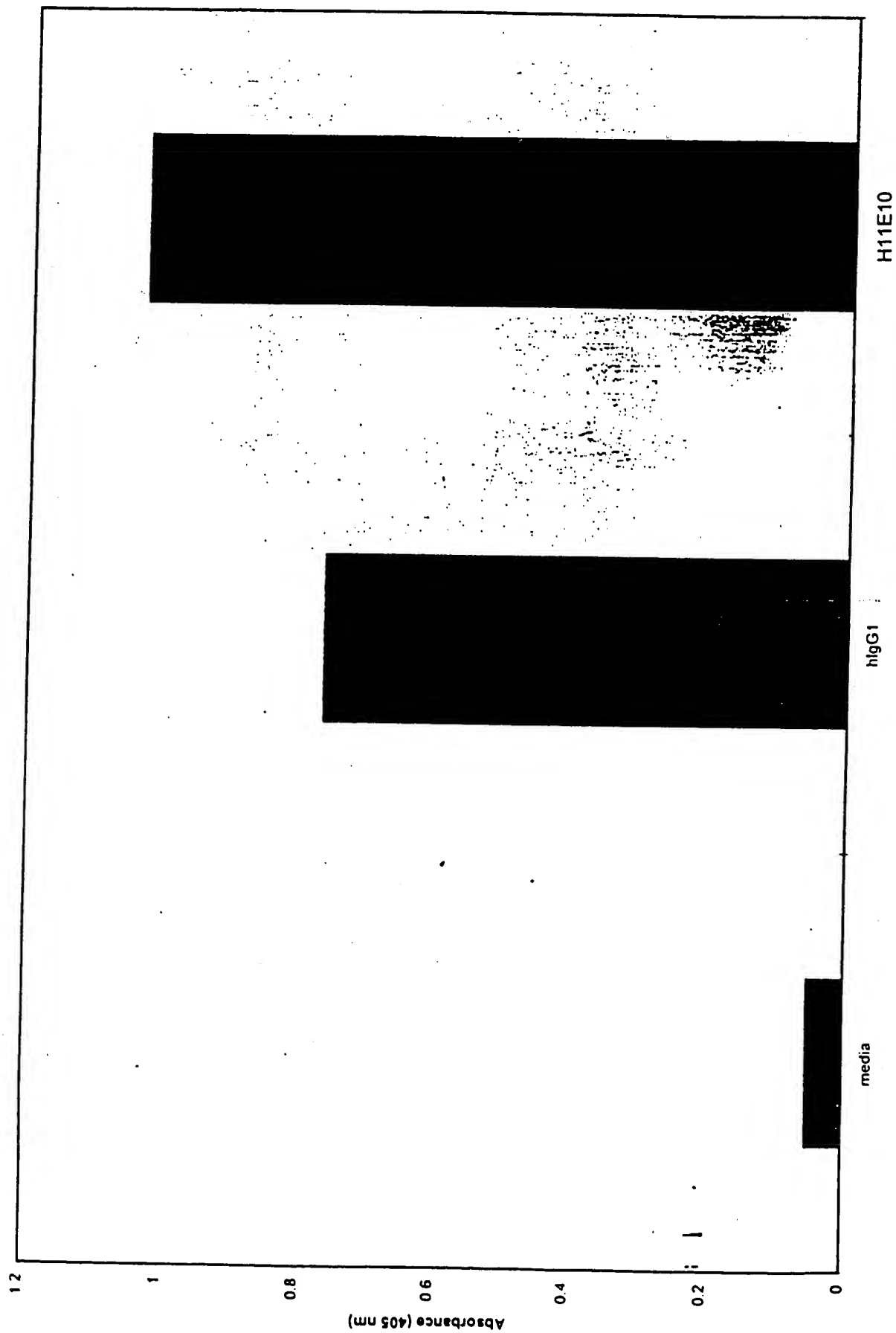
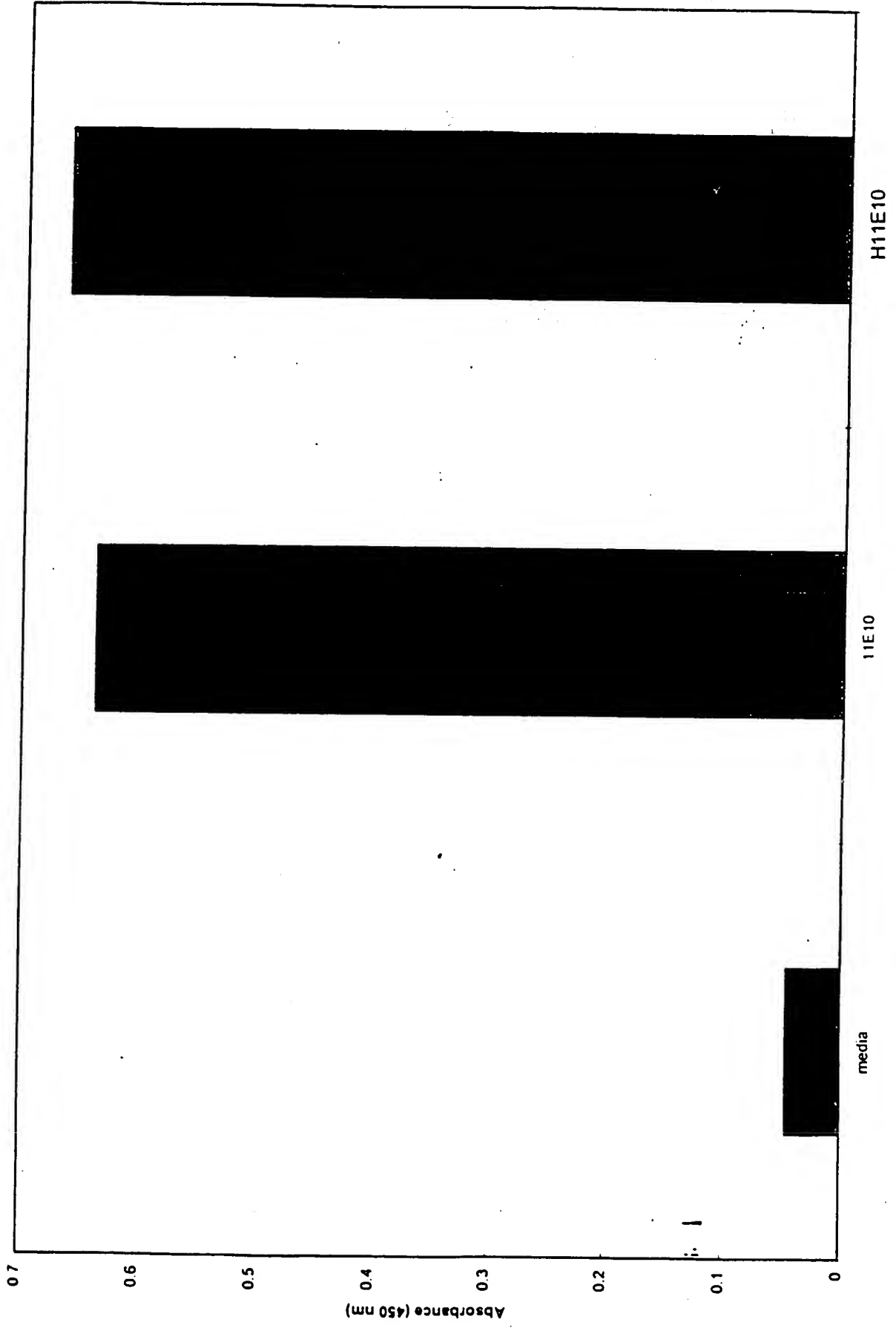


FIGURE 11
Anti-Stx2 Activity ELISA



pCDNA3mut.LCPL.LCVK construction:

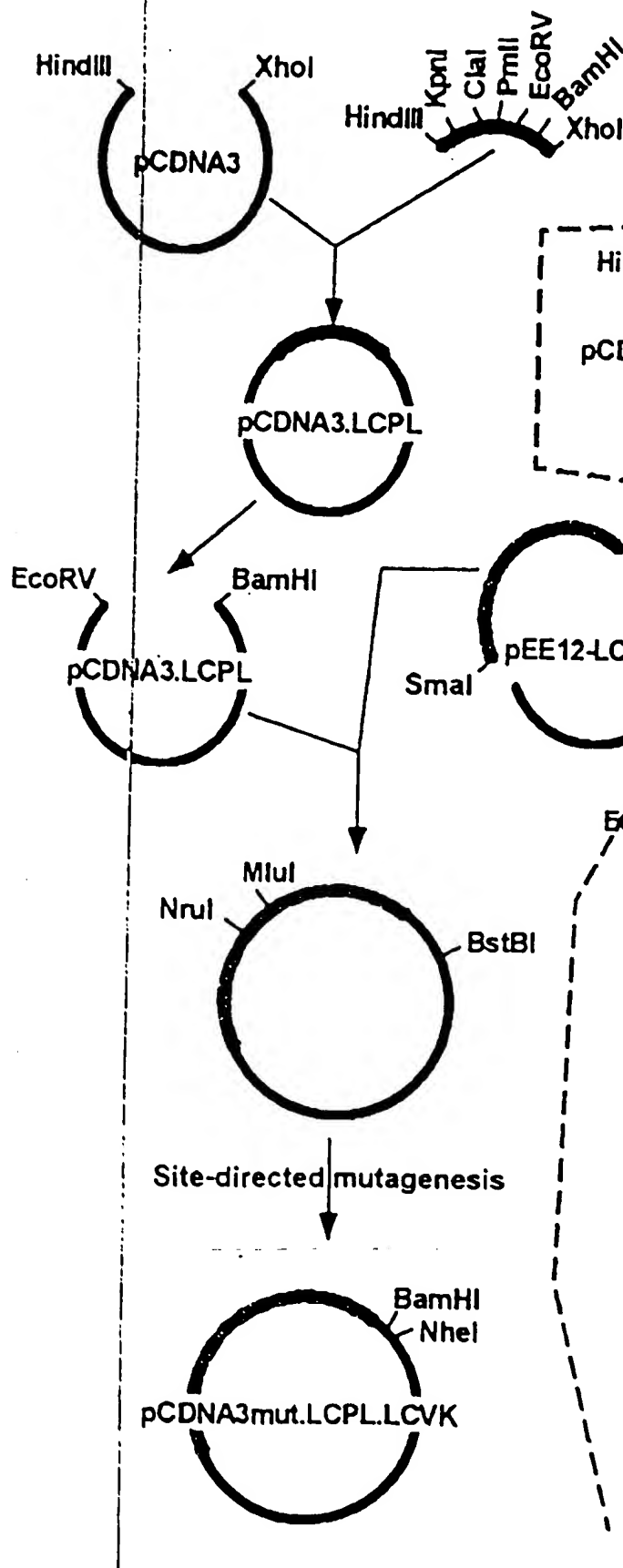


FIGURE 12

pCDNA3mut.HCPL.HCV2b construction:

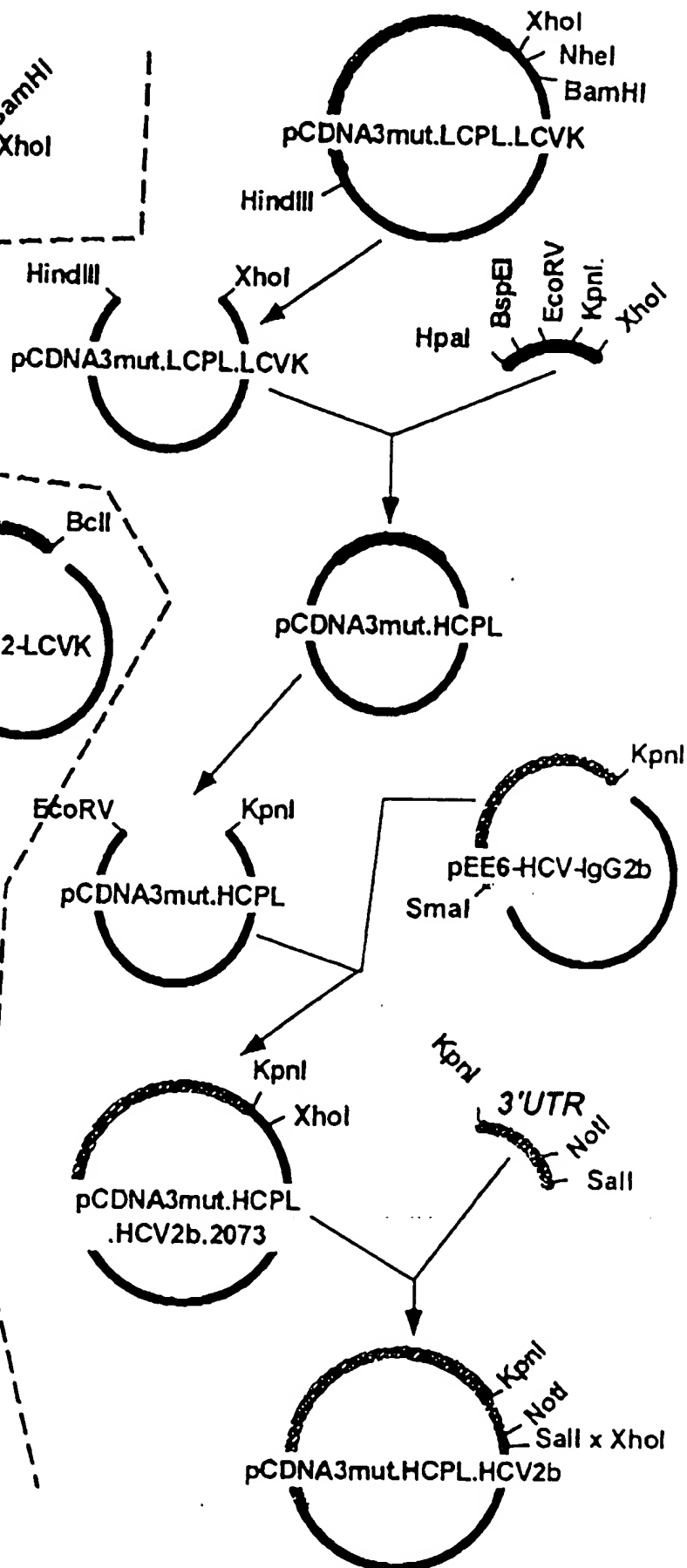


FIGURE 13

pSUNs, 9:Kappa and pSUN10, 10:IgG1 constructions:

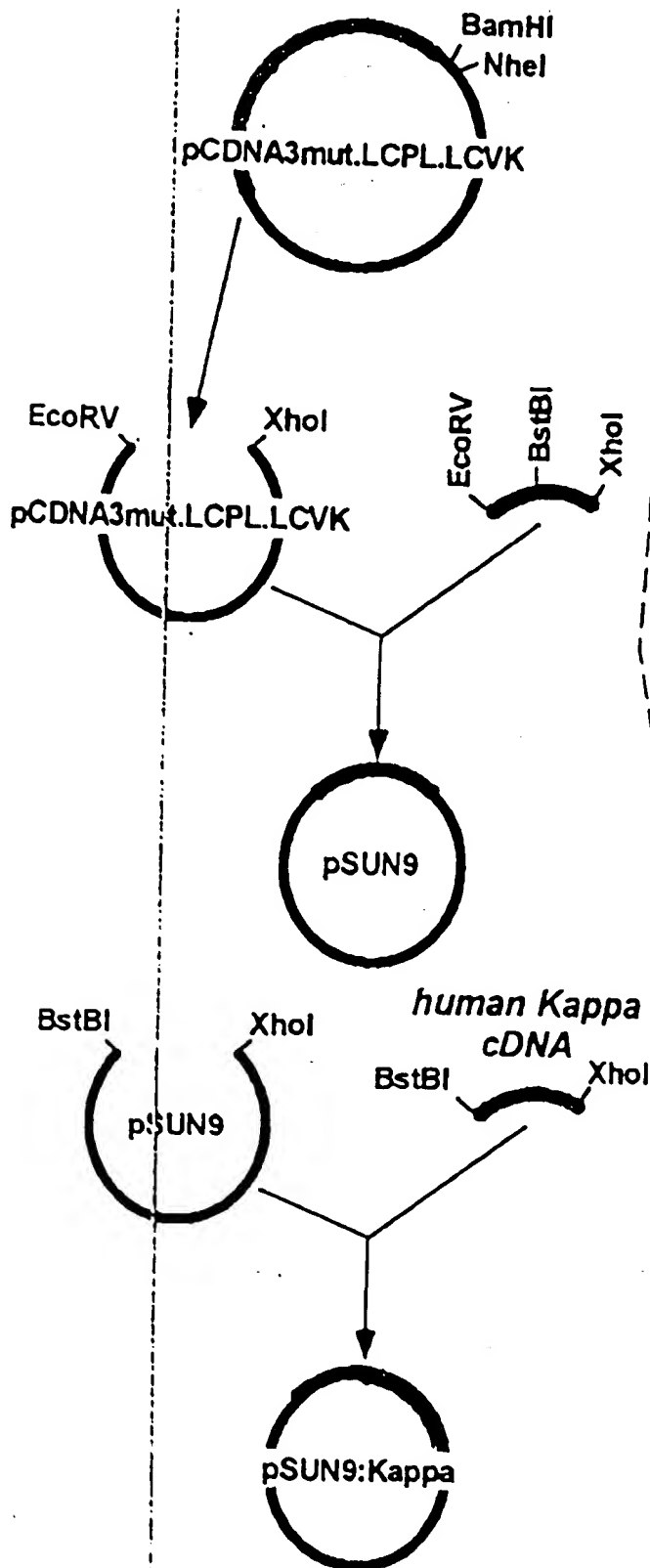


FIGURE 14

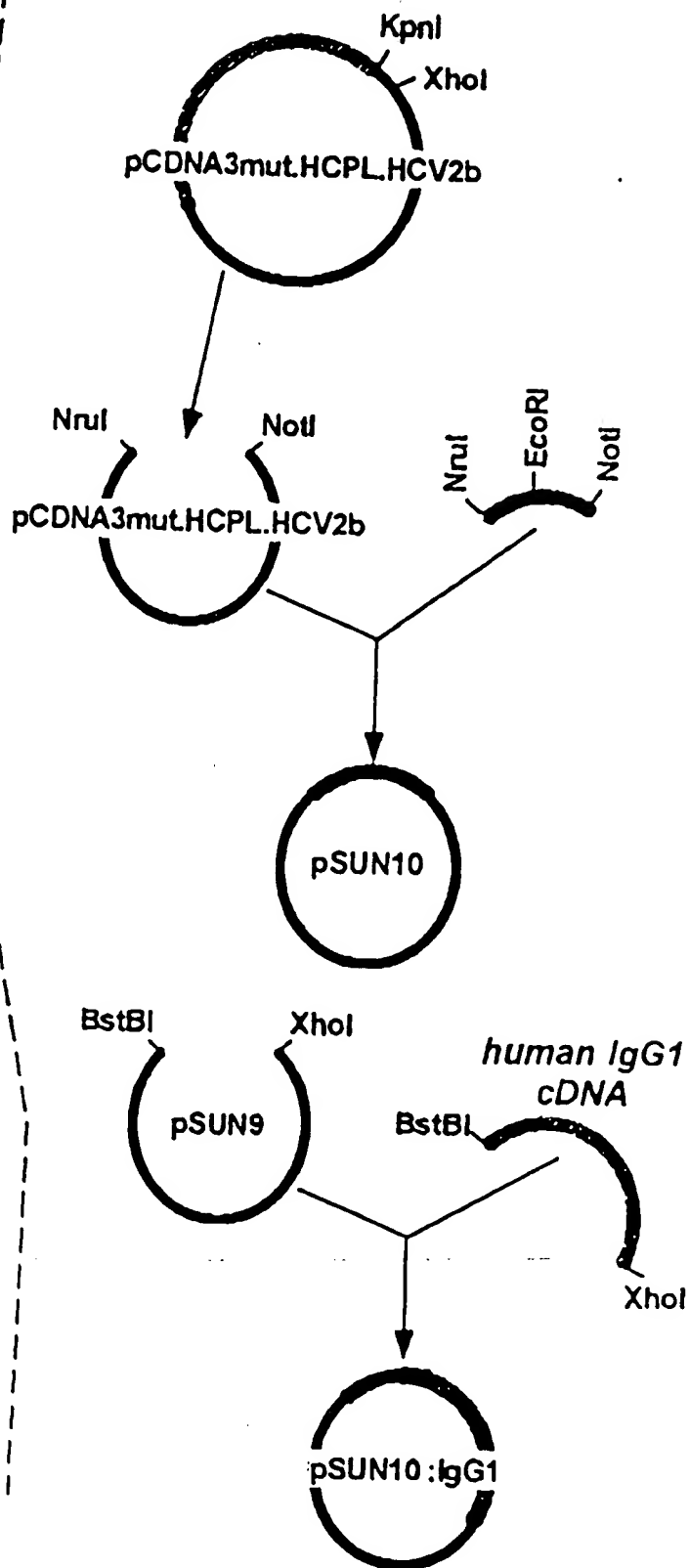


FIGURE 15

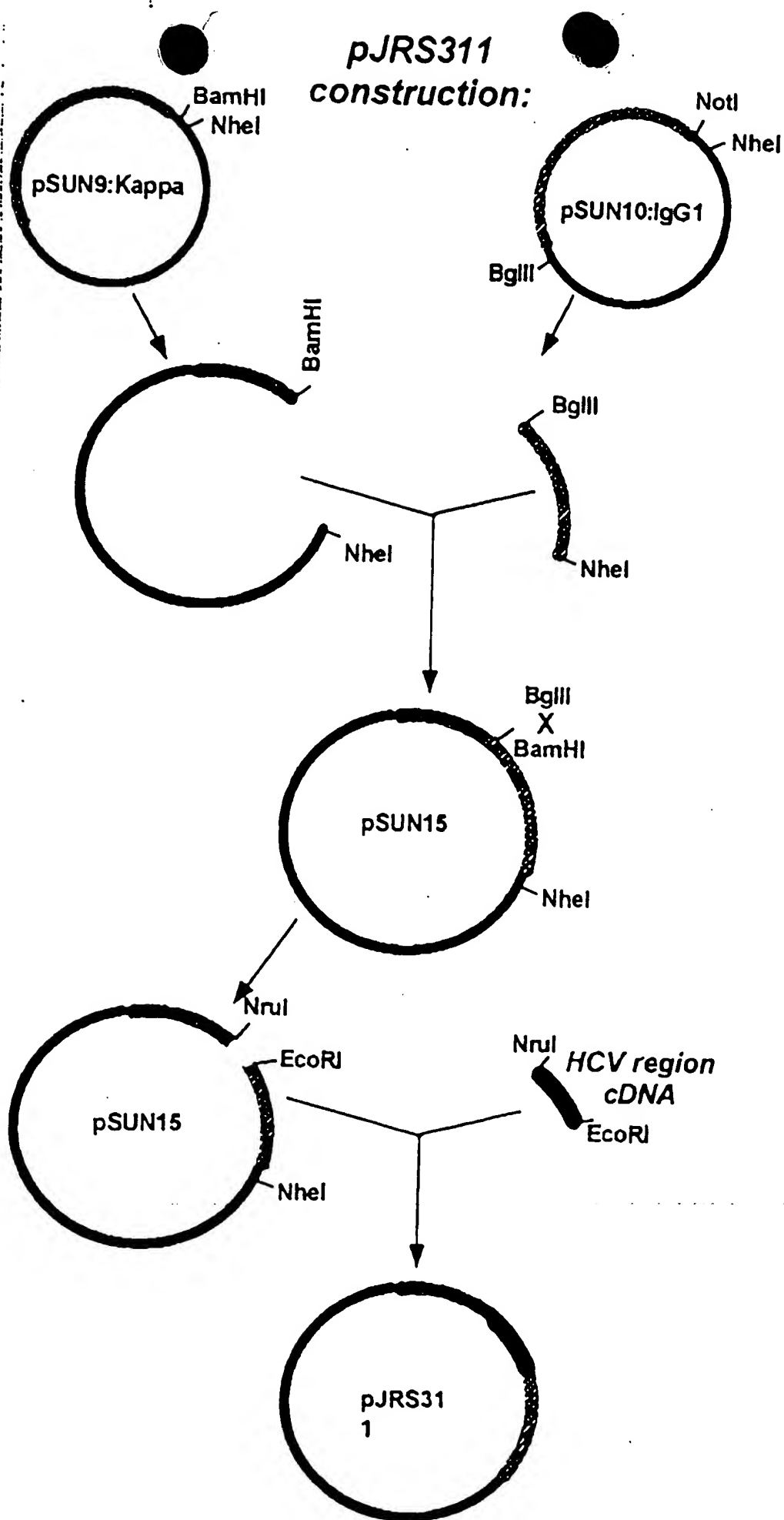


FIGURE 16

**pJRS315
construction:**

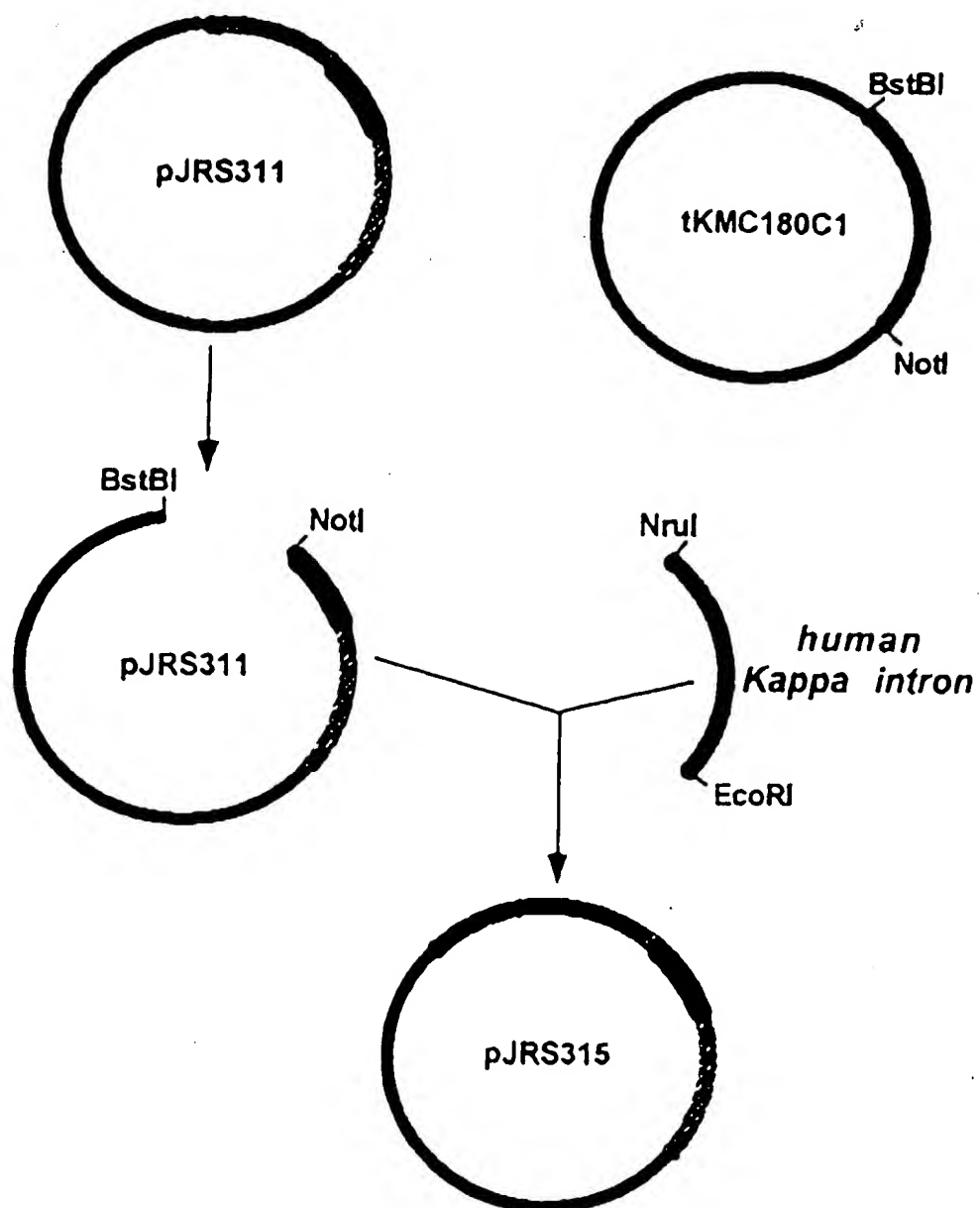


FIGURE 17